Note: Variable names will be added to this document in the near future.

Group 1 Assays

Cholesterol: Total cholesterol is measured in EDTA plasma using a cholesterol oxidase method (Roche Diagnostics, Indianapolis, IN 46250) on a Roche COBAS FARA centrifugal analyzer at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). This method incorporates cholesterol esterase and peroxidase in the reagent and monitors cholesterol oxidation at 500 nm upon conversion of 4-aminoantipyrine to quinoneimine. This enzymatic method is standardized with a serum standard prepared in our laboratory and frozen at -70°C. The assigned value of this standard is set by replicate Abell-Kendall cholesterol analysis performed by a CDC/NHLBI Cholesterol Reference Method Laboratory Network laboratory. The calibration of this assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends reference range of <200 mg/dL. The laboratory CV is 1.6%.

HDL-Cholesterol: HDL-cholesterol is measured in EDTA plasma using the cholesterol oxidase cholesterol method (Roche Diagnostics) after precipitation of non-HDL-cholesterol with magnesium/dextran. This method is standardized as described for the cholesterol assay; and calibration of the assay is regularly monitored by the CDC/NHLBI Lipid Standardization Program. The NCEP program recommends reference range of >40 mg/dL. Measurements are made at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The laboratory CV is 2.9%.

Triglyceride: Triglyceride is measured in EDTA plasma using Triglyceride GB reagent (Roche Diagnostics, Indianapolis, IN 46250) on the Roche COBAS FARA centrifugal analyzer. This assay performs an automated glycerol blank by taking a spectrophotometric reading after endogenous glycerol has reacted and before lipase is added to release the glycerol from the triglyceride. This method is calibrated with a frozen serum standard prepared in our laboratory and frozen at -70°C. We have assigned this calibrator by comparison to CDC reference materials. The accuracy and precision The NCEP program recommends reference range of <150 mg/dL. Measurements are made at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The laboratory CV is 4.0%

LDL-Cholesterol Calculated: LDL-cholesterol is calculated in plasma specimens having a triglyceride value <400 mg/dL using the formula of Friedewald et al. (Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502). The NCEP program recommends reference range of <100 mg/dL. Measurements are made at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN).

Serum creatinine: Serum creatinine is measured by rate reflectance spectrophotometry using thin film adaptation of the creatine amidinohydrolase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY 14650) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The reference range in

adult females is 0.4 – 1.1 mg/dL and in adult males is 0.5 – 1.2 mg/dL. The laboratory CV is 2.2%.

Glucose: Serum glucose is measured by rate reflectance spectrophotometry using thin film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY 14650) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The adult reference range is 60 – 115 mg/dL. The laboratory CV is 1.1%.

Group 2 Assays

DNA Isolation: DNA is isolated at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN) from packed EDTA and citrate cells that are frozen at –70°C. The DNA extraction and purification method uses sodium dodecylsulfate cell lysis followed by a salt precipitation method for protein removal using commercial Puregene® reagents (Gentra System, Inc., Minneapolis, MN 55447). A mean yield of 39.1 µg DNA/mL packed cell was obtained, and DNA was of high quality (mean purity A260/280=1.77) and high molecular weight as determined by gel electrophoresis.

Urinary Creatinine: Urinary creatinine is measured using the Vitros 950IRC instrument (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY) at the Clinical Chemistry Laboratory at Fletcher Allen Health Care (Burlington, VT). Thin film technology is used to quantitatively measure creatinine via a colorimetric reaction. The assay range is 0.05 – 16.50 mg/dl, with a CV range of 2.5 – 2.9%. The normal reference range is 0.7 – 1.5 mg/dl.

Urinary Albumin: Urinary albumin is determined using the Array 360 CE Protein Analyzer (Beckman Instruments, Inc., Drea, CA) at the Clinical Chemistry Laboratory at Fletcher Allen Health Care (Burlington, VT). This system utilizes a nephelometer to measure the rate of light scatter formation resulting from an immunoprecipitation reaction. The minimum detectable level of albumin is 0.2 mg/dl. The normal reference range is <1.9 mg/dl.

Total Homocysteine (tHcy): Plasma tHcy is measured by a fluorescence polarization immunoassay (IMx Homocysteine Assay, Axis Biochemicals ASA, Oslo, Norway) using the IMx Analyzer (Abbott Diagnostics, 100 Abbott Park Rd, Abbott Park, Illinois 60064) at the Biochemical Genetics Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The method is based on the enzymatic conversion of free homocysteine to S-adenosyl-L-homocysteine, which is subsequently detected by a competitive immunoassay. The assay range is 0.5-50 µmol/L with a laboratory CV range of 3.8 – 5.1%. The reference range on fasting plasma is 4.0 - 12.0 µmol/L.

Interleukin-6 (IL-6): IL-6 is measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The lower detection limit is <0.0.094 pg/mL with a detection range of 0.156-10.0 pg/mL. A monoclonal anti-IL6 antibody is coated on the plastic support and a polyclonal anti-IL6 antibody is used as the sandwich antibody. The amount

of IL-6 bound is determined by a color reaction. The expected normal range per the manufacturer is 0.24 to 12.5 pg/mL. The laboratory CV for this assay is 6.3%.

C-reactive protein (CRP): CRP is measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL) at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). This instrument utilizes a particle enhanced immunonepholometric assay to determine CRP. Polystyrene particles are coated with monoclonal antibodies to CRP that agglutinate in the presence of antigen (CRP) to cause an increase in the intensity of scattered light. The increase in scattered light is proportional to the amount of CRP in the sample. The assay range is 0.175 – 1100 mg/L. Expected values for CRP in normal, healthy individuals are ≤ 3 mg/L. Intra-assay CVs range from 2.3 – 4.4% and inter-assay CVs range from 2.1 – 5.7%.

Chlamydia pneumoniae (*C. pneumoniae*): IgG antibodies to *C. pneumoniae* are detected in serum using a microimmunofluorescent antibody (MIF) assay employing a two stage sandwich procedure for the qualitative and semi-quantitation detection of IgG antibodies to *C. pneumoniae* (Focus Technologies, Cypress, CA). The method is performed at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). Positive reactions appear as bright apple-green fluorescent elementary bodies with a background matrix of yolk sac. Fluorescence is graded as follows: 2 to 4+: moderate to intense apple-green fluorescence; 1+: definite, but dim fluorescence; negative: no fluorescence. A positive test is one in which fluorescence is 1+ or greater.

Insulin: Insulin is determined by a raidoimmunoassay method using the Linco Human Insulin Specific RIA Kit (Linco Research, Inc., St. Charles, MO 63304). This assay utilizes ^{125}I-labeled Human Insulin and a Human Insulin antiserum to determine the level of insulin. The lower limit of sensitivity is 2 U/L with a laboratory CV of 4.9%. Measurements are made at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The reference range on fasting serum is <20 mU/L .

Fibrinogen: Fibrinogen antigen is measured using the BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). The amount of fibrinogen present in the sample is quantitatively determined by immunochemical reaction. Complexes formed between antigen and antibody molecules scatter light passing through the sample. The intensity of the scattered light is proportional to the concentration of the antigen (fibrinogen) in the sample. Expected values for fibrinogen in normal, healthy individuals are 180 – 350 mg/dl. The intra-assay and inter-assay CVs are 2.7% and 2.6%, respectively.

Factor VIII: Factor VIII levels are determined by measuring the clot time of a sample in factor VIII deficient plasma in the presence of activators utilizing the Sta-R analyzer (STA-Deficient VIII; Diagnostica Stago, Parsippany, NJ). The results are given as percent factor VIII, with reported normal plasma range of factor VIII in the adult population between 60 and 150%.

D-Dimer: Fibrin fragment D-dimer is measured using an immuno-turbidimetric assay (Liatest D-DI; Diagnostica Stago, Parsippany, NJ) on the Sta-R analyzer (Diagnostica Stago, Parsippany, NJ). This assay is performed at the Laboratory for Clinical Biochemistry Research (University of

Vermont, Burlington, VT). The assay utilizes microlatex particles to which specific antibodies have been attached. In the presence of the antigen (D-dimers), the antibody-coated latex particles agglutinate to form aggregates that absorb more light. This increase in light absorption is a function of the antigen level present in the test sample. The normal reference range is 0.22 to 4.0 ug/mL, with expected normal values <0.4 μg/ml.

Boneu B, Aptel I, Nguyen F, Canbus JP, Thirion C, Amiral J, Boccalon H, Elias A (1997) Liatest D-Di. A new fast assay to determine D-Dimers, has performances comparable to classical ELISA for diagnosis of deep vein thrombosis. Thromb Haemost, supp. 159, abstract PD 651, June 1997.

Plasmin-α_2-antiplasmin complex (PAP): PAP is measured in an assay that detects only plasmin in complex with α_2-antiplasmin, and not free plasmin or α_2-antiplasmin. As such, it is an excellent marker of active plasmin generation. This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The assay is a two-site ELISA that utilizes two monoclonal antibodies (Holvoet, et al, 1986). The analytical CV for this assay is 1.7%. The expected normal range is 2.1 to 5.8 nM. Reagents for this assay are generously provided by Drs. Désiré Collen and Paul Declerck (University of Leuven, Leuven, Belgium).

Holvoet P, deBoer A, Verstreken M, Collen D (1896) An enzyme-linked immunosorbent assay (ELISA) for the measurement of plasmin-alpha-2-antiplasmin complex in human plasma - application to the detection of in vivo activation of the fibrinolytic system. Thromb Haemost 56:124-127.

NMR Lipids: Individual lipoprotein subclasses are measured using the NMR LipoProfile-II spectral analysis process by Dr. Jim Otvos (LipoScience, Inc.; Raleigh, NC). The instrument employs proton NMR spectroscopy to measure the particle concentrations of 11 subclasses of VLDL, LDL, and HDL. In addition, calculated values for mean VLDL, LDL, and HDL particle size and estimates of total and VLDL triglycerides and HDL cholesterol are provided. The CVs for the particle concentrations of VLDL, LDL, and HDL are 4% or less. CVs for individual subclasses (large VLDL, medium VLDL, small VLDL, IDL, small LDL, medium small LDL, very small LDL, large HDL, small HDL) are under 10%. CVs for IDL and medium HDL are 27.5%. CVs for mean VLDL, LDL, and HDL mean particle size are 2.0% or less. For calculated total triglycerides, VLDL triglycerides, and HDL cholesterol, CVs range from 1.1-1.4%.

Group 3 (MESA 1000) Assays

Soluble Intercellular Adhesion Molecule-1 (sICAM-1): sICAM-1 is measured by an ELISA assay (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN). This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). sICAM-1 is sandwiched by an immobilized monoclonal antibody and the enzyme-linked monoclonal antibody. The amount of ICAM-1 present is determined by colorimetric reaction. The laboratory CV is 5.0%, with a healthy reference mean of 326 ± 89 ng/mL. The minimum detectable level is < 0.35 ng/ml with an assay range of 2.73 – 49.55 ng/ml.

von Willebrand factor (vWf): vWf is measured by an immunoturbidimetric assay on the Sta-R analyzer (liatest vWF; Diagnostica Stago, Parsippany, NJ). The assay utilizes latex particles to which specific antibodies have been attached. In the presence of antigien (vWF) the particles agglutinate to form aggregates, which absorb more light. This increase in absorbance is proportional to the vWF present in the test sample. This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The results are presented as percent vWF, with an expected normal range of 50-160%. Intra-assay CV is 3.7% and inter-assay CV is 4.5%.

Plasminogen Activator Inhibitor-1 (PAI-1): The PAI-1 assay was originally developed by Dr. Désiré Collen and colleagues (DeClerck, et al, 1988), and is sensitive to free PAI-1 (both latent and active) but not PAI-1 in complex with tissue plasminogen activator. The assay is a two-site ELISA performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The Laboratory for Clinical Biochemistry Research has extensive experience with this assay (Macy, et al, 1993), having used it in over 6,000 epidemiological participants to date. Reagents are generously provided by Dr. Collen (Leuven, Belguim). The analytical CV for this assay is 3.5%. The significant diurnal change in PAI-1 levels and the potential for contamination by platelets, makes attention to the details of blood drawing particularly important (Macy, et al, 1993; Tracy and Bovill, 1995). The expected normal range is 5 -66 ng/mL.

DeClerck P, Alessi M, Verstreken M, Kruithof E, Juhan-Vague I, Collen D (1988) Measurement of plasminogen activator inhibitor 1 (PAI-1) in biological fluids with a murine monoclonal antibody based enzyme-linked immunosorbent assay. Blood 71:220-225.

Macy E, Meilahn E, DeClerck P, Tracy R (1993) Sample preparation for plasma measurement of plasminogen activator inhibitor-1 antigen in large population studies. Arch Path Lab Med 177:67-70.

Tracy R, Bovill E (1995) Plasminogen activator inhibitor-1. In E Beutler, M Lichtman, B Coller, T Kipps (Eds.), Williams Hematology, pps. L110—L111. New York; McGraw Hill.

HDL Subfractions: HDL subfraction concentrations are determined in plasma using the Lipoprint HDL System (Quantimetrix Corporation, Redondo Beach, CA) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The HDL subfractions are separated by high resolution polyacrylamide gradient gel electrophoresis on the basis of their molecular size. The gels are scanned by a densiotometer, and a public domain software program (NIH Image Version 1.62) identifies 8 major HDL subfractions according to their migration distance (Rf) relative to the albumin fraction. The percent and concentration (mg/dL) of each fraction is calculated. The laboratory CV's for the 8 major fractions range from 6.3 to 21.8%. The software also allows for the option of isolating three HDL subfractions. Laboratory CV's for the three fractions are 9.2, 7.2 and 17.4%, respectively.

LDL Subfractions: LDL subfraction concentrations are determined in plasma using the Lipoprint HDL System (Quantimetrix Corporation, Redondo Beach, CA). The LDL subfractions are separated by high resolution polyacrylamide gradient gel electrophoresis on the basis of their molecular size. A typical Lipoprint profile consists of 1 VLDL band, 3 Midbands, up to 7 LDL bands and 1 HDL band. The gels are scanned by a densiotometer, and a public domain software program (NIH Image Version 1.62) identifies fractions by their mobility (Rf) using VLDL as the starting reference point and HDL as the leading reference point . The percent and concentration (mg/dL) of each fraction is calculated. Reported CV's range from 1.2 – 7.3% for HDL, LDL and VLDL fractions, 2.9 – 11.1% for midband subfractions, and 1.7 – 17.9% for LDL subfractions. Measurements are made at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN).

Cholesteryl Ester Transfer Protein (CETP) Mass: CETP mass is measured in serum by a sandwich enzyme immunoassay (Wako CETP Test; Wako Chemical USA, Inc., Richmond, VA) method at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). Serum samples are pretreated with detergent to release CETP from lipoproteins. The monoclonal antibody coated to the solid phase and the horse radish peroxidase (HRP)-labeled monoclonal antibody reacts with the released CETP. The activity of HRP bound to the solid phase is proportional to the CETP mass in the sample. The laboratory CV for this assay is 11.1%. The reference range is reported to be 1.92 ± 0.57 μg/mL.

Cholesteryl Ester Transfer Protein (CETP) Activity: CETP activity is determined in plasma using a CETP Activity Kit (Roar Biomedical, Inc., New York, NY) that includes donor (without apo-A1) and acceptor particles. The method is performed at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). Incubation of donor and acceptor with a CETP source results in the CETP mediated transfer of fluorescent neutral lipid from donor molecule to a VLDL acceptor molecule. The fluorescence of the reaction increases as the fluorescent neutral lipid is removed from the core of the donor molecule to the acceptor. CETP activity is reported in nmol/mL/hr. The laboratory CV for this assay is 13.3%.

Anti-Human Heat Shock Protein 60 (Hsp60): Anti-human Hsp60 antibodies (IgG, IgA and IgM) are measured by an ELISA assay (StressXpress™ Anti-Human Hsp60 (total) ELISA Kit; Stressgen, Victoria, BC, Canada) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). Anti-human Hsp60 antibodies bind to recombinant human Hsp60 coated to the solid phase. The captured anti-human Hsp60 antibodies are detected with hydrogen peroxidase conjugated goat polyclonal antibody specific for human IgG, IgA and IgM antibodies. The reported kit sensitivity is 2.88 ng/mL. The laboratory CV for this assay is 18.8%.

Remnant-like Particle-Cholesterol (RLP-C): RLP-C is measured using the RLP®-Cholesterol Immunoseparation Assay (POLYMEDCO, Inc.; Cortlandt Manor, NY) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). This method uses immunoseparation techniques to isolate a population of remnant lipoproteins that have the same physical and chemical properties of β-VLDL—these particles are referred to as remnant-like particles (RLP). The remnant particles are separated from LDL, Lp(a), nascent VLDL and HDL by mouse monoclonal antibodies to human apolipoproteins A-1 and B-100

conjugated to sepharose-4B beads. The remnant particles present in the unbound fraction are measured by assaying the cholesterol (remnant lipoprotein cholesterol) by an enzymatic assay using the Roche Cobas Mira centrifugal analyzer (Roche Diagnostics, Indianapolis, IN 46250). The laboratory CV range for this assay is 5.5% - 9.3%.

Red Blood Cell (RBC) Membrane Fatty Acids: The RBC are separated from EDTA plasma at the Field Centers, an equal volume of acid citrate-dextrose preservative solution is added, and the mixture is sent to the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN) for fatty acid analysis. The RBC are lysed, the fatty acids are extracted from the RBC ghosts and quantitated by gas-liquid chromatography. Quantitation is achieved by measuring peak area relative to an internal standard. The fatty acid profile includes approximately 29 fatty acids from 12:0 through 24:1ω9. Our laboratory CV is 6% for most fatty acids analyzed.

Herpes Simplex Virus (HSV) IgG: Serum IgG antibodies to herpes simplex virus (HSV) type 1 and type 2 are detected using an indirect enzyme immunoassay (Diamedix Immunosimplicity® HSV 1 & 2 Test Kit, Diamedix Corporatio;, Miami, Florida) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). EU/mL (ELISA) of each specimen is determined by comparison to absorbance values obtained on a predetermined calibrator. EU/mL of <16.0 is interpreted as negative for anti-HSV 1 & 2 IgG; EU/mL of 16.0-19.9 EU/mL obtained on two determinations is equivocal for anti-HSV 1 & 2 IgG; and ≥20.0 EU/mL is interpreted as positive for anti-HSV 1 & 2 IgG.

Cytomegalovirus (CMV) IgG: Serum IgG antibodies to cytomegalovirus (CMV) are detected using an indirect enzyme immunoassay (Diamedix Immunosimplicity® CMV IgG Test Kit, Diamedix Corporation; Miami, Florida) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). EU/mL (ELISA) of each specimen is determined by comparison to absorbance values obtained on predetermined standards. EU/mL of <8.0 is interpreted as negative for anti-CMV IgG; EU/mL of 8.0-9.9 obtained on two determinations is equivocal for anti-CMV IgG; and EU/mL of ≥10.0 is interpreted as positive for anti-CMV IgG.

Helicobactor pylori (*H. pylori*): Serum IgG antibodies to *H. pylori* antigen are detected using an indirect enzyme immunoassay (Diamedix Immunosimplicity® *H. pylori* IgG Test Kit, Diamedix Corporation, Miami, Florida) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The Index Value of each specimen is determined by comparison to absorbance value obtained on a cut-off calibrator. Index Value of <0.90 is interpreted as no detectable antibodies to *H. pylori*; Index Value of 0.90 - 1.09 obtained on two determinations is equivocal for IgG antibodies to *H. pylori*; and Index Value of ≥1.10 is interpreted as positive for *H. pylori* antibody.

Antibody to Hepatitis A Virus (anti-HAV): Total serum antibodies to hepatitis A virus (anti-HAV) are detected using the IM®x HAVAB qualitative microparticle enzyme immunoassay (MEIA) (Abbott Laboratories; Abbott Park, IL) at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN). The rate of each specimen is determined and compared to a cutoff rate obtained on each run by a calibrator, as described by

the manufacturer. Values greater than the Cufoff rate are considered nonreactive by the criteria of the IMx HAVAB assay; values less than the Cufoff rate are considered reactive by the criteria of the IMx HAVAB assay.

Interleukin-2 soluble receptor α chain (IL-2 sRα): IL-2 sRα is measured by ultra-sensitive ELISA (Quantikine Human IL-2 sRα Immunoassay; R&D Systems, Minneapolis, MN). This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT).The assay plate is precoated with a monoclonal antibody specific for IL-2 sRα and an anti-IL-2 sRα polyclonal antibody is used as the sandwich antobody. The amount of IL-2 sRα is determined colorimetrically. The lower detection level is 10 pg/ml and the detection range is 78.1 – 5000 pg/ml. The assay CV ranges from 4.6 – 7.2%.

Tumor Necrosis Factor-α soluble receptor 1 (sTNF-R1): sTNF-R1 is measured using an ultra-sensitive ELISA assay (Quantikine Human sTNF RI Immunoassay; R&D Systems, Minneapolis, MN). A monoclonal antibody specific for sTNF-R1 is coated on the assay plate and a polyclonal anti-TNF-R1 antibody is used as the sandwich assay. The amount of receptor is determined by a colorimetric reaction. This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The laboratory CV for this assay is 5%. The lower detection level is 1-3 pg/ml and the detection range is 7.8–500 pg/mL. The normal range for TNF-R1 in serum is 479-1966 pg/mL.

Soluble Thrombomodulin (sTM): sTM is measured by enzyme immunoassay using a monoclonal antibody to TM as the capture antibody (Asserachrom Thrombomodulin, Diagnostica Stago; Asnières-sur-Seine, France). A second horse radish peroxidase labeled monoclonal antibody is used to detect bound sTM. The amount of sTM in the sample is determined colorimetrically. This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The laboratory CV for this assay is 12%. The detection range is 4–60 ng/ml and the lower detection limit is 2 ng/ml.

Tissue factor pathway inhibitor (TFPI): TFPI is measured by enzyme-linked sandwich ELISA using a polyclonal anti-TFPI antibody as the capture antibody (Imubind Total TFPI ELISA Kit, American Diagnostica, Inc.; Stamford, CT). TFPI is detected using a biotinylated monoclonal antibody specific for the Kunitz domain 1 of TFPI. Binding of streptavidin conjugated horse radish peroxidase to the TFPI-antibody complex and the addition of substrate provide a colorimetric determination of the amount of TFPI in the sample. This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The assay range is 15.20–200.0 ng/mL and the lower limit of detection is 0.180 ng/mL. The expected range of TFPI in normal, healthy individuals is 75–120 ng/mL (mean value of 89.5 ng/mL). Intra-assay and inter-assay CVs range from 6.2–7.1% and 5.5–7.3%, respectively.

Thrombin Activatable Fibrinolysis Inhibitor (TAFI): TAFI, also known as procarboxypeptidase-B and procarboxypeptidase-U, is measured using the ELISA for Thrombin Activatable Fibrinolysis Inhibitor from Affinity Biologicals Inc. (Ancaster, Ontario, Canada). This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The assay range is 0.10–3.30 μg/ml and the minimum detectable level is 0.41

μg/ml. The normal range for TAFI is reported to be 5.4-10 μg/ml. CVs for this assay range from 3.5%-4.2%.

Soluble Tissue Factor (sTF): sTF is measured by an enzyme-linked immunoassay that employs an anti-TF monoclonal capture antibody (Imubind Tissue Factor ELISA Kit, American Diagnostica, Inc.; Stamford, CT). sTF is detected using a biotinylated antibody fragment that specifically recognizes bound sTF. Binding of streptavidin conjugated horse radish peroxidase to the sTF-antibody complex and the addition of substrate provide a colorimetric determination of the amount of sTF in the sample. This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The assay range is 50.0–1000 pg/mL and the minimum detectable limit is 10 pg/mL sTF. No normal range for sTF has been established. The laboratory CV for this assay is 14.6%.

Soluble E-Selectin (sE-Selectin): sE-selectin, also known as endothelial leukocyte adhesion molecule-1 (ELAM-1) and CD62E, is measured using a high sensitivity quantitative sandwich enzyme (Parameter Human sE-Selectin Immunoassay; R&D Systems, Minneapolis, MN) at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The sE-Selectin assay utilizes two antibodies directed against different epitopes on the sE-Selectin molecule. The amount of sE-selectin bound is determined colorimetrically. The minimum detectable level of sE-Selectin is typically <0.1 ng/mL and the assay range is 0.47 – 10.52 mg/mL. The expected normal range in serum is 29.1 – 63.4 ng/mL. Intra-assay and inter-assay CVs range from 4.7 – 5.0% and 5.7 – 8.8%, respectively.

Matrix Metalloproteinse-9 (MMP-9): MMP-9, also known as gelatinase B, is measured by a high sensitivity quantitative sandwich enzyme immunoassay (Quantikine Human MMP-9 (total) Immunoassay; R&D Systems, Minneapolis, MN). A monoclonal antibody specific for MMP-9 is coated on the assay plate and an enzyme-linked polyclonal anti-MMP-9 antibody is used as the sandwich assay. The amount of antigen (MMP-9) is determined by a colorimetric reaction. This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The minimum detectable level of MMP-9 is typically <0.156 ng/mL and the assay range is 0.31 – 20 ng/mL. Expected normal values range from 169 – 705 ng/mL. Intra-assay and inter-assay CVs range from 1.9 – 2.9% and 6.9 – 7.9%, respectively. The assay results may be affected by the presence of platelets in samples. MMP-9 is released upon platelet activation and platelets in the sample may cause variable and irreproducible results.

Matrix Metalloproteinase-3 (MMP-3): MMP-3, also know as stromelysin-1, is measured by an ultra-sensitive, solid-phase sandwich ELISA using a polyclonal antibody specific for both the pro- and active forms of MMP-3 (Quantikine Human MMP-3 (total) Immunoassay; R&D Systems, Minneapolis, MN). This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The minimal detectable level of MMP-3 ranges from 0.002 - 0.045 ng/mL (mean limit of 0.009 ng/mL) with an assay detection range of 0.16 - 10 ng/mL. The expected normal range of MMP-3 in serum is 2.10 – 64.4 ng/mL. Intra-assay and inter-assay CVs range from 5.7 - 6.4% and 7.0 - 8.6%, respectively.

CD40 Ligand (CD40L): CD40L, also known as CD154, gp39, TNF-related activation protein (TRAP), and T-cell B-cell activating molecule (TBAM), is measured by an ultra-sensitive

quantitative sandwich enzyme immunoassay technique (Quantikine Human soluble CD40 Ligand Immunoassay; R&D Systems, Minneapolis, MN). This assay is performed at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The minimum detectable level of CD40L ranges from 2.1 – 10.1 pg/ml (mean of 4.2 pg/ml) and the assay range is 62.5 – 4000 pg/mL. The expected normal range in serum is 675 – 38,373 pg/mL. Intra-assay and inter-assay CVs range from 4.5 – 5.4% and 6.0 – 6.4%, respectively. Assay results may be affected by the presence of platelets in samples. CD40L is present in platelet granules and released upon platelet activation. Platelets in the sample may cause variable and irreproducible results.

Oxidized LDL (oxLDL): oxLDL is measured by competitive ELISA (Mercodia Oxidized LDL ELISA, Mercodia AB, Uppsala, Sweden) utilizing a specific murine monoclonal antibody to oxLDL and copper-oxidized LDL as a competitive ligand. This assay was developed by Dr. Paul Holvoet and colleagues (Holvoet et al, 1998) and is performed in Dr. Holvoet's laboratory (University of Leuven; Leuven, Belgium). The intra-assay and inter-assay CVs range from 5.5-7.3% and 4.0-6.2%, respectively. The total CV for the assay ranges from 7.4-8.3%.

Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D (1998) Oxidized LDL an dmalondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation 98:1487-1494.

Malondialdehyde-modified LDL (MDA-modified LDL): MDA-modified LDL is measured by an ELISA developed by Dr. Paul Holvoet and colleagues (Holvoet P et al, 1995) and is performed in Dr. Holvoet's laboratory (University of Leuven; Leuven, Belgium).

Holvoet P, Perez G, Zhao, Z, Brouwers E, Berner H, Collen D (1995) Malondialdehyde-modified low density lipoproteins in patients with atherosclerotic disease. J Clin Invest 95:2611-2619.

Hemoglobin A_1C (HbA_1C): HbA_1C is measured in EDTA blood that is diluted at the Field Centers in a sample preparation vial containing an aqueous solution of EDTA and potassium cyanide (HbA1c Sample Preparation Kit, Bio-Rad, Hercules, CA 94547). Specimen from the prep vial is analyzed on the Tosoh A_1c 2.2 Plus Glycohemoglobin Analyzer (Tosoh Medics, Inc., San Franciscok CA 94080) using an automated high performance liquid chromatography method. Reference range is 4.3 – 6.0% with a laboratory CV range of 1.4 - 1.9%. Measurements are made at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN).

ATP-Binding Cassette Transporter A1 (ABCA1) Genotypes: Two common polymorphisms of the ABCA1 gene are genotyped using polymerase chain reaction (PCR) and detection by restriction fragment length polymorphism (RFLP): 1) The –477C/T polymorphism is a change of cytosine to thymine in the promoter region of the ABCA1 gene and is detected by *AciI* digestion of a 351bp amplified PCR product. 2) The 1051G/A (R219K) polymorphism is a guanine to adenine transition in exon 7 resulting in an arginine to lysine amino acid change. The

polymorphism is detected by *StyI* digestion of a 433bp amplified PCR product. All genotypes are reported as wildtype (normal), heterozygous, or mutant for each respective polymorphism.

MESA Sub-study Assays

F2 Isoprostanes: n = 436